



Menadione: Role in cancer prevention and methods of analysis

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ABSTRACT

Menadione (vitamin K₃) is a water-insoluble vitamin and an analogue of vitamin K. Its use as an anti-cancer drug is increasing day by day. It produces its anti-tumor effect without causing any harm to the normal cells. Various methods have been developed for the analysis of menadione in pure form, pharmaceutical products and biological fluids which includes spectrophotometry, chromatography, potentiometry, colorimetry, electrochemical, chemiluminescences and polarographic methods. Of all the methods HPLC methods have been found to give more reliable and sensitive results. Fluorimetric methods are more promising as compared to the spectrophotometric methods for the assay of menadione. Certain polarographic methods have been found to give better results for the biological fluids. Chemiluminescence methods can be used for the assay of the vitamin without facing any interference.

Keywords: Menadione, anti-cancer, spectrophotometry, HPLC



INTRODUCTION

Menadione (vitamin K₃), a provitamin [1], is a synthetic analog of 1,4-naphthoquinone having a methyl group at position 3 [2] (Figure 1). It is formed as a result of catabolism after intestinal absorption of vitamin K and also appears in urine [3]. It occurs as pale yellow crystalline powder, odorless, which is insoluble in water and freely soluble in toluene. It is light sensitive and incompatible with oxidizing agents. It should not be given to the neonates and during the third trimester of pregnancy as it can cause the development of hemolytic anemia, kernicterus and hyperbiliribemia to the infants. However it can be safely used during lactation [4]. Studies have been conducted related to the chemical and physical properties and the different analysis techniques of menadione [5]. Vitamin K is essentially required for the proteins which are responsible for blood clotting [6]. It also has major role in bone mineralization [7] and developing nervous system [8]. Deficiency of vitamin K results in easy bleeding like nose bleeding, gum bleeding and blood in stool and urine. However deficiency of this vitamin is not common in healthy people as vitamin K is abundantly found in a number of foods [9]. Soya bean oil, olive oil, green leafy vegetables and canola oil are the major sources of vitamin K [10]. There is no reported toxicity

related to vitamin K₁ and K₂, but menadione can interfere with body's antioxidant causing cell membrane damage [11]. Menadione is not prescribed for vitamin K deficiency as it induces liver toxicity and hemolytic anemia in infants when given through parenteral route [9]. The effects of vitamin K are inhibited by large doses of vitamin A and vitamin E [12]. Vitamin K antagonizes the effect of warfarin [13]. The fetal vitamin K synthesis may be disturbed by drugs like rifampicin, isoniazid, warfarin and certain anticonvulsants [14].

ANTI-CANCER PROPERTIES OF MENADIONE

The new finding regarding menadione has diverted the attention of the physicians towards the role of this vitamin in treating cancer at different stages [16]. Out of all the vitamin K analogues, K₂ and K₃ has shown such properties. Studies have shown that vitamin K₃ exhibits its anticancer effect at relatively high doses [17]. A new anticancer drug (imatinib mesylate) in combination with vitamin K [18] started suppressing the growth of the cancer cells found in the lungs [19] and similar effects were observed in the cancer cells of liver and bladder [20,21]. In vitro studies have shown that menadione has proved itself to be an emerging drug for the treatment of solid tumors like in lung

cancer but its use in gastrointestinal cancers has not shown any promising results when given in combination with the traditional chemo drugs [22, 23]. Vitamin K kills the tumor cells without causing any harm to the normal cells and they do this by stimulating oxidative stress [24]. The mechanism by which menadione exhibits its anticancer properties can be summarized by these three points: it inhibits the formation of the new blood vessels which may be helpful in the rapid growth of the cancer tissues [25], it hinders the cells in proliferating by interfering with the microtubules [19] and also it prevents the enzymes responsible for the DNA formation [26]. It has been found that menadione exhibits a mechanism which has been given the name of 'oncosis'. Vitamin K₃ activates the ischemic cell death and the cancerous cells are specifically prone to it [1]. Combination of menadione with vitamin C has proved to be very effective against cancer cells, the combination causes the cancer cells to split open and emptying their contents [27]. Research has been conducted revealing the role of menadione and ascorbic acid in treating prostate cancer [28], and the dose adjusted was 50 mg/day and 5000 mg/day respectively for those who did not respond to the standard treatment [29]. A group of researcher has also find out that ascorbic acid causing the reduction of menadione is helpful in killing the breast cancer cells [30].

ANALYTICAL METHODS FOR THE ASSAY OF MENADIONE

Various analytical methods have been used for the assay of menadione and its salts in pharmaceutical preparation and biological fluids. These methods are reviewed in the following sections. Figure 2 represents the graphically the analytical methods used for the analysis of menadione.

Spectrometric methods: Two spectrophotometric methods have been proposed for the determination of menadione and its sodium bisulfate salt in pharmaceutical products. The methods have been found to be rapid, simple and sensitive. One method involves the formation of a blue colored product on reacting menadione with 3-methyl-2-benzothiazolinone hydrazone hydrochloride, the product have the λ_{max} of 625 nm. Another method is carried out by reacting menadione with resorcinol resulting in the formation of a red colored compound showing the maximum absorption at 520 nm. The methods can be applicable within the concentration range of 0.4-16 $\mu\text{g ml}^{-1}$ and 1-24 $\mu\text{g ml}^{-1}$ respectively [31]. A group of researchers has conducted a validated method for the determination of menadione in plasma by liquid chromatography-tandem mass spectrometry.

The method has been found to be selective and sensitive and has shown good precision and reproducibility. It is based on the derivatization of menadione with 3-mercaptopropionic acid and identified by mass spectra. The method has been proved to be 33 times better than the analysis performed on the underivatized compound and the quantification limit for the vitamin is 0.03 ng ml^{-1} [32]. A two derivative spectrophotometric method has been developed for the determination of menadione sodium bisulphate by converting it into menadione using sodium carbonate. The method can be easily applied to the pharmaceutical preparations. The quantitative determination required 350 nm and 355nm for the first- and second –order derivative method respectively [33]. A group of researchers have performed the analysis of menadione in bulk and pharmaceutical preparations by reacting it with 3-methyl-2-benzothiazolone hydrozone in the presences of ferric chloride. No interferences were observed and the absorption was detected at the wavelength between 650-670 nm [34]. Methods have also been developed to determine menadione in vitamin mixtures [35].

Fluorimetric methods: A method has been proposed for the fluorimetric determination of menadione. Menadione sodium bisulfate (water-soluble salt) is extracted with methanol and with the help of sodium carbonate is converted into a fat-soluble vitamin. After analysis with RP-HPLC the vitamin is subjected to fluorescent and is converted into its 1,4-dihydroxy analogue. The concentration range of 1-100 mg of menadione showed the fluorescent response. Average standard deviation was found to be 5.5% and all the recoveries were more than 90% [36]. A group of workers conducted the fluorimetric determination of menadione by HPLC with post-column derivatization. The sodium bisulfate salt of the vitamin in animal feed was determined at the concentrations of 0.02 $\mu\text{g/gm}$. The recovery was found to be 94.4 +/- 6.8% [37]. A successful method for the determination of menadione sodium bisulfate in pharmaceutical formulations by spectrofluorometry has been developed. The sample was mixed with NaOH, Na₂SO₃ and acetone in a T connector. The photochemical reactor was attached to a 6-W mercury lamp at an emission wavelength of 459 nm and an excitation wavelength of 336 nm [38].

High performance liquid chromatographic methods: A rapid and simple reversed phase HPLC method has been proposed for the analysis of menadione in biological fluids (blood serum and urine) along with retinol, menaquinone, cholecalciferol, α -tocopherol, α -tocopherol acetate,

δ -tocopherol and phylloquinone. The Phenomenex Luna C18 column was used and the flow rate was adjusted at 1.3 ml/min. the detection was conducted at 280nm and the detection limit was found to be in the range of 1.4-6.6ng per 20 microl injected samples. The recovery ranges for the blood serum were within 95-97.6% and for urine were 94.02-95.8% [39]. An accurate, simple and sensitive HPLC procedure for the analysis of menadione has been conducted. The vitamin obtained from the plasma was extracted using n-hexane and methanol was added to the extraction solution to prevent loss of vitamin. The method employed the detection at 265 nm. The recovery was 82.4 +/- 7.69% [17]. Study has been conducted utilizing the technique of HPLC using C 30 column along with the development of fluorescence detection to determine menadione in urine. 95% methanol and 0.55% aqueous solution of 5% DI H₂O were utilized as the mobile phase. The study has shown linear calibration curve and has been found to be reproducible and sensitive [40]. Another accurate and successful HPLC method has been developed for the determination of vitamin K₁ and K₃ found in the facial skin cream. The detection has been performed at 333 nm using C 18 column. Linear calibration curve has been observed for the concentration of 0.2-1.0 mg/ml. the method has been found to be rapid and simple and can be used successfully for the determination of menadione in commercial creams [41]. A group of workers performed the analysis of five fat-soluble vitamins including menadione, retinyl acetate, cholecalciferol, α -tocopherol and α -tocopherol acetate in feed. The film coating of the tablets was destroyed with the help of enzyme, ethanol was utilized for the extraction of vitamins and for the purpose of purification, Oasis HLB was used. Methanol: water (98:2 v/v) was used at the mobile phase while the detection was carried out at 230 and 265 nm. The method has been found to be linear, accurate and repeatable [42]. A method has been proposed for the determination of fat-soluble vitamins: menadione, retinoic acid, retinal, retinol, vitamin D₂ and D₃ utilizing the technique of RP-HPLC. The vitamins were extracted from the plasma of rabbit. The UV detection was made at 245 nm and the mobile phase was composed of methanol: ethanol (85:15 v/v) with 0.1% triethylamine. For extraction different solvents were used for different vitamins. The method has been found to be linear and showed 40% recovery for each vitamin [43]. Studies have also been conducted for the analysis of vitamin K₃ in animal feed [44,45], multivitamin formulations [46,47] and in minerals and vitamin supplements [48].

Polarographic methods: Study has been conducted to carry out differential pulse

polarographic assay of menadione found in plasma. The material is extracted from plasma and then treated with ether and finally dissolved in methanol. The method has been reported to give linear calibration curve [49]. Group of workers have proposed a quantitative polarographic procedure for the analysis of vitamin K₃. Almost the whole quantity of the vitamin is recovered completely. The non-aqueous polarographic medium consists of petroleum ether extract of the feed containing menadione [50].

Chemiluminescence method: A successful method has been proposed where the chemiluminescence is produced by the bisulfate given off by the menadione on reaction with Ce (IV). The concentration range was within 0.01-10 μ g/ml and the RSD was found to be 3.45% [51]. A simple, selective and sensitive method has been proposed for the determination of menadione and its conjugates. The compounds are reacted with dithiothreitol and luminal is used as a chemiluminescence probe to detect oxygen. No interferences were detected and linearity was observed in the range of 5-240 for menadione and its conjugates (52).

Potentiometric methods: Favorable results than the standard spectrophotometric method has been claimed to by the researchers who have performed a potentiometric method for the analysis of menadione. Polyvinyl chloride matrix is used as the membrane sensor interacting with menadione anions in the presences of bathophenanthroline nickel (II) and iron (II). The method has shown linear response for the concentrations of 10⁻¹-10⁻⁵ M, fast response time, low detection limit and good stability and selectivity coefficient [53].

Electrochemical methods: It has been found that menadione exhibits redox reaction on a carbon electrode in the presences of 0.1M H₃PO₄ with the potential separation of 343 mV. This property of the vitamin has been utilized for its analysis by developing a voltametric method. The surfactants (cationic, anionic and non-anionic) produce negative effect on the potential separation. The sensitivity of the method has been found to be $(8.6 \pm 0.2) \times 10^3 \mu\text{A } \mu\text{M}^{-1}$ [54]. A method has been proposed for the analysis of menadione. Menadione gives a well-defined polarographic catalytic wave in the presences of KIO₃ maintained at pH 4.7. The peak potential was observed at -0.955 V. the concentration ranges within 4.0×10^{-8} - 2.0×10^{-6} mol/L. KIO₃ has contributed to the sensitivity of the method [55].

Colorimetric methods: Study has been done for the determination of menadione calorimetrically.

Menadione forms purple complex with chloranilic acid in the presences of ethanol-dioxane mixture which can be measured at 510 nm. The recovery of the method has found to be 97.6% within the concentration range of 0.2-1.1 mg% [56]. Another colorimetric method has been proposed for the

determination of menadione in fodder preparations. The vitamin obtained from the carotenoids is extracted with the help of benzene. The method has been found to be suitable for pharmaceutical preparations also [57].

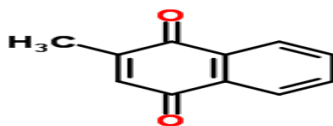


Figure 1: Structure of Menadione

Table 1: Selected Physico-Chemical properties of Menadione [15]

Empirical formula	C ₁₁ H ₈ O ₂
Chemical name	2-methyl-1,4-dihydronaphthalene-1,4-dione
Molecular weight	172.18
Melting point	107°C
Density	1.225 g/cm ³
Solubility in water	160 mg/L (at 30 °C)
pKa (acidic)	12.94

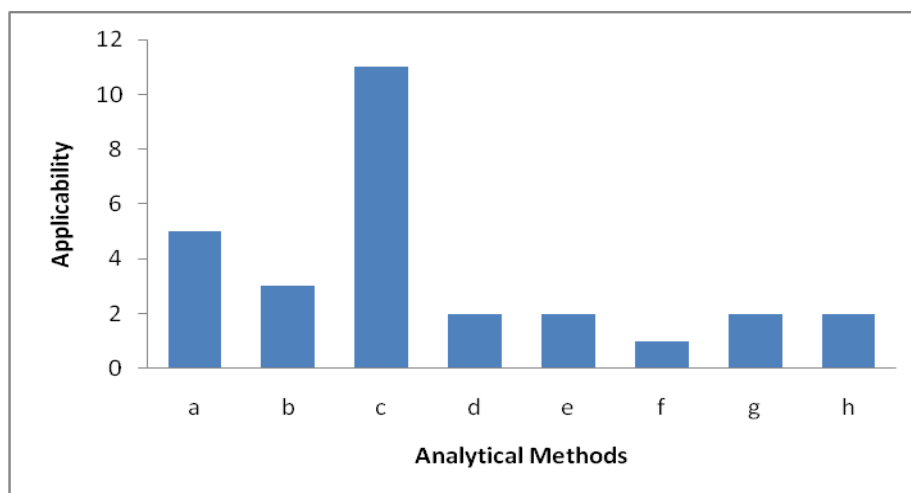


Figure 2: Application of the analytical methods for the determination of menadione in active, pharmaceutical preparations and biological fluids: a. spectrometric method; b. fluorimetric methods; c. HPLC methods; d. polarographic methods; e. chemiluminescence methods; f. potentiometric methods; g. electrochemical methods and h. colorimetric methods.

REFERENCES

1. Scott GK et al. Vitamin K3 (menadione)-induced oncosis associated with keratin 8 phosphorylation and histone H3 arylation. *Mol Pharmacol* 2005; 68(3): 606-615.
2. Castro FA et al. Cytotoxicity Mechanism of Two Naphthoquinones (Menadione and Plumbagin) in *Saccharomyces cerevisiae*. *PLoS ONE* 2008; 3(12): e3999.
3. Thijssen HH et al. Menadione is a metabolite of oral vitamin K. *Br J Nutr* 2006; 95(2): 260-266.
4. von Kries R et al. Vitamin K1 content of maternal milk: influence of the stage of lactation, lipid composition and vitamin K1 supplements given to the mother. *Pediatr Res* 1987; 22: 513-517.
5. Hassan GS. Menadione. *Profiles Drug Subst Excip Relat Methodol* 2013; 38: 227-313.
6. Brody T. *Nutritional Biochemistry*. 2nd ed. San Diego: Academic Press; 1999.
7. Shearer MJ. The roles of vitamins D and K in bone health and osteoporosis prevention. *Proc Nutr Soc* 1997; 56(3): 915-937.
8. Tsaion KI. Vitamin K-dependent proteins in the developing and aging nervous system. *Nutr Rev* 1991; 57(8): 231-240.
9. Olson RE. Vitamin K. In: *Modern Nutrition in Health and Disease*. 9th ed. Shils M, Olson JA, Shike M, Ross AC, Eds; Lippincott Williams & Wilkins, Baltimore, 1999; pp. 363-380.
10. Food and Nutrition Board, Institute of Medicine. *Vitamin K In: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. National Academy Press, Washington DC, 2001; pp. 162-196.
11. Ferland G. Vitamin K. In: *Present Knowledge in Nutrition*. 9th ed. Volume 1. Bowman BA, Russell RM, Eds; ILSI Press, Washington DC, 2006; pp. 220-230.

12. Booth SL et al. Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. *Am J Clin Nutr* 2004; 80(1): 143-148.
13. Booth SL, Centurelli MA. Vitamin K: a practical guide to the dietary management of patients on warfarin. *Nutr Rev* 1999; 57(9 Pt 1): 288-296.
14. Hendler SS, Rorvik DR, eds. *PDR for Nutritional Supplements*. Montvale: Medical Economics Company, Inc; 2001.
15. ChemicalProductProperty_EN_CB4494726.htm, <http://www.chemicalbook.com> (Accessed August 25, 2014)
16. Louise M et al. Menadione: Spectrum of anticancer activity and effects on nucleotide metabolism in human neoplastic cell lines. *Biochem Pharmacol* 1991; 41(9): 1283-1292.
17. Hu OY et al. Determination of anticancer drug vitamin K3 in plasma by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* 1995; 666(2): 299-305.
18. Yokoyama T et al. Combination of vitamin K2 plus imatinib mesylate enhances induction of apoptosis in small cell lung cancer cell lines. *Int J Oncol* 2005; 26(1): 33-40.
19. Acharya BR et al. Vitamin K3 disrupts the microtubule networks by binding to tubulin: a novel mechanism of its antiproliferative activity. *Biochemistry* 2009; 48(29): 6963-6974.
20. Kassouf W et al. Vitamins C and K3 sensitize human urothelial tumors to gemcitabine. *J Urol* 2006; 176(4 Pt 1):1642-7.
21. Wei G et al. Vitamin K enhancement of Sorafenib-mediated HCC cell growth inhibition in vitro and in vivo. *Int J Cancer* 2010; 127(12): 2949-2958.
22. Tetef M et al. Mitomycin C and menadione for the treatment of advanced gastrointestinal cancers: a phase II trial. *J Cancer Res Clin Oncol* 1995; 121(2): 103-106.
23. Tetef M et al. Mitomycin C and menadione for the treatment of lung cancer: a phase II trial. *Invest New Drugs* 1995; 13(2): 157-162.
24. Verrax J et al. Targeting cancer cells by an oxidant-based therapy. *Curr Mol Pharmacol* 2008; 1(1): 80-92.
25. Taper HS. Altered deoxyribonuclease activity in cancer cells and its role in non toxic adjuvant cancer therapy with mixed vitamins C and K3. *Anticancer Res* 2008; 28(5A): 2727-2732.
26. Matsubara K et al. Inhibitory effects of vitamin K3 on DNA polymerase and angiogenesis. *Int J Mol Med* 2008; 22(3): 381-387.
27. Verrax J et al. The association of vitamins C and K3 kills cancer cells mainly by autophagy, a novel form of cell death. Basis for their potential use as adjuvants in anticancer therapy. *Eur J Med Chem* 2003; 38(5): 451-457.
28. Lasalvia-Prisco E et al. Serum markers variation consistent with autophagy induced by ascorbic acid-menadione in patients with prostate cancer. *Med Oncol* 2003; 20(1): 45-52.
29. Tareen B et al. A 12 week, open label, phase I/IIa study using apatone for the treatment of prostate cancer patients who have failed standard therapy. *Int J Med Sci* 2008; 5(2): 62-67.
30. Beck R et al. Menadione reduction by pharmacological doses of ascorbate induces an oxidative stress that kills breast cancer cells. *Int J Toxicol* 2009; 28(1): 33-42.
31. Nagaraja P et al. Spectrophotometric methods for the rapid determination of menadione and menadione sodium bisulphite and their application in pharmaceutical preparations. *J Pharm Biomed. Anal* 2002; 28 (1): 161-168.
32. Ruijuan Liu et al. A novel liquid chromatography-tandem mass spectrometry method for determination of menadione in Human plasma after derivatization with 3-mercaptopropionic acid. *Talanta* 2014; 128: 51-57.
33. Fatma Demirkaya-Miloglu et al. Determination by converting Menadione sodium bisulphite to Menadione in pharmaceutical preparation by two derivative spectrophotometric methods. *Eur Int J Sci Technol* 2013; 2: 89-96
34. Sastry CSP et al. Spectrophotometric determination of menadione and menadione sodium bisulfite in pharmaceutical preparations. *Int J Pharma* 1987; 39(1-2): 137-140.
35. Hewala II. Determination of two binary vitamin mixtures using difference and derivative spectrophotometry. *Analyt Lett* 2006; 26(10): 2217-2237.
36. Billedeau SM. Fluorimetric determination of vitamin K3 (menadione sodium bisulfite) in synthetic animal feed by high-performance liquid chromatography using a post-column zinc reducer. *J Chromatogr* 1989; 472(2): 371-379.
37. Speek AJ et al. Fluorimetric determination of menadione sodium bisulphite (vitamin K3) in animal feed and premixes by high-performance liquid chromatography with post-column derivatization. *J Chromatogr* 1984; 301(2): 441-447.
38. He Q et al. Determination of menadione sodium bisulfite in pharmaceutical preparations by flow-injection on-line photochemical spectrofluorometry. *Anal Sci* 2001; 17(10): 1209-1212.
39. Chatzimichalakis PF et al. Development of a validated liquid chromatography method for the simultaneous determination of eight fat-soluble vitamins in biological fluids after solid-phase extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; 805(2): 289-96.
40. Al Rajabi A et al. Measurement of menadione in urine by HPLC. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; 878(26): 2457-2460.
41. De Orsi D et al. Identification and quantitation of vitamins K1 and K3 in cosmetic products for facial skin protection. *J Cosmet Sci* 2008; 59(6): 459-67.
42. Xue X et al. Simultaneous determination of five fat-soluble vitamins in feed by high-performance liquid chromatography following solid-phase extraction. *J Chromatogr Sci* 2008; 46(4): 345-350.
43. Po ES et al. Simultaneous chromatographic analysis of eight fat-soluble vitamins in plasma. *J Biochem Biophys Methods* 1997; 34(2): 99-106.
44. Schneiderman MA et al. Determination of menadione in an animal feed using supercritical fluid extraction and HPLC with electrochemical detector. *J Chromatogr Sci* 1988; 26(9): 458-462.
45. Manz U, Maurer R. A method for the determination of vitamin K3 in premixes and animal feedstuffs with the aid of high performance liquid chromatography. *Int J Vitam Nutr Res* 1982; 52(3): 248-252.
46. Sadlej-Sosnowska N et al. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography. *J Chromatogr* 1986; 357(1): 227-232.
47. Gabriela K, Elzbieta LA. Determination of the content of vitamin K in complex vitamin preparations by the HPLC method. *Act Pol Pharm* 2002; 59(2): 93-97.
48. Ranft K, Rückemann H. Methods for the determination of vitamins by means of HPLC. IV. Determination of vitamin K3 in vitamin premixes and mineral supplements. *Z Lebensm Unters Forsch* 1978; 167(3): 150-151.
49. Akman SA et al. Differential pulse polarographic determination of plasma menadione. *Anal Biochem* 1984; 141(2):488-493.
50. Onrust H, Wöstmann B. A polarographic determination of vitamin-K3 (2-methyl-1,4-naphthoquinone) in prepared feeds. *Recueil des Travaux Chimiques des Pays-Bas* 1950; 69(10), 1207-1216.
51. Huang Y et al. Chemiluminescent determination of menadione bisulfate. *Anal. Lett* 1999; 32(14): 2789-2798.
52. Elgawish MS et al. Development and validation of the first assay method coupling liquid chromatography with chemiluminescence for the simultaneous determination of menadione and its thioether conjugates in rat plasma. *Chem Res Toxicol* 2013; 26(9): 1409-1417.
53. Rizk NMH. Potentiometric determination on menadione (Vitamin K3). *Microchimica Acta* 2002; 138(1-2): 53-58.
54. Guzel Ziyatdinova et al. Electrochemical reduction and quantification of menadione in sodium dodecyl sulfate micellar media. *J Solid State Electrochem* 2013; 17(10): 2679-2685.
55. Song J et al. Determination of menadione based on its polarographic catalytic wave in the presence of potassium iodinate. *Anal. Lett* 2001; 34 (1): 1677-1688.
56. Ajali UO et al. Colorimetric determination of menadione sodium bisulfate with chloranilic acid. *Ind J Pharm Sci* 2003; 65(5): 492-495.
57. van Koetsveld E.E. A colorimetric method for the determination of vitamin K3 in fortified fodders. *Recueil des Travaux Chimiques des Pays-Bas* 1950; 69(10): 1217-1222.